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UNPUBLISHED PRELIMINARY DATA

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(2) QUARTERLY PROGRESS REPORT NO. 11

TO NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

(1) RADIOISOTOPIC BIOCHEMICAL PROBE FOR EXTRATERRESTRIAL LIFE

(NASA CONTRACT NO. NASr-10)

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Gilbert V. Levin et al

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RADIOISOTOPIC BIOCHEMICAL PROBE FOR EXTRATERRESTRIAL LIFE

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SUMMARY

Four remote field tests, conducted at sites selected for adverse environmental conditions, were successful in detecting life within very short periods. The sites tested were: Orange, Virginia which provided a hard, clay soil of high iron content at the surface; Sheep Mountain in White Mountains, California which provided an altitude of 12,200 feet, an area above the tree line, and extremely rocky terrain; Death Valley, California which provided an arid, sandy region; and the Salton Sea area of the Imperial Valley, also in California. This last site provided a surface soil which was hard, rocky, and of high salt content. In spite of some minor operational and mechanical problems, metabolic responses were positive, rapid, and distinct. The antimetabolite was also generally effective.

Several in situ determinations which were made, demonstrated the desirability of investigating Gulliver modifications to permit the examination of CO_2 evolution from organisms in soil without moving the soil into an instrument.

Changes have been made to the basal medium. The medium used currently has been designated M9 and contains soil extract and glycine-1-C^{14} in addition to the constituents of the M8 medium previously used. DL-tyrosine-1-C^{14} is still being evaluated. DL-1-alanine-1-C^{14} and a commercial, nonradioactive liver extract were evaluated, but felt to make no significant contribution when used with the current basal constituents.

Studies are continuing in an effort to enhance the growth of pure cultures and mixed populations from soils. The addition of radioactive and cold glucose in various concentrations up to 1.0% has not been adequate to produce increased growth responses from soil populations in the basal salts medium. However, Escherichia coli responded to the addition of glucose with increased growth. Other growth factors are currently being investigated for their utilization for increased growth responses.

The Bard Parker preparation continues to be an effective antimetabolite. Mercuric chloride, Argyrol, and acrolein were tested but were less effective against mixed populations in soil than the Bard Parker.

Additions of the following pure cultures were made to the test collection: Lactobacillus plantarum, Clostridium perfringens, Aerobacter aerogenes, Sphaerotilus natans, Leptothrix cholodnii, Leptothrix discophora, Leptothrix lopholea, and Leptothrix pseudo-ochracea.

Field test sites have provided distinctive types of soils: an iron-rich soil, Orange, Virginia; a rock-pebble soil from the White Mountain range in California; sand from Death Valley, California; and a highly saline soil from the Salton Sea flats in California. In addition, a saline soil from the edge of the Atlantic Ocean in Florida has been included. All the soils have produced positive responses in life detection tests with M9.

The isolation of colonies from soils in the test collection for purposes of investigating the sources of the general response obtained from a heterogeneous soil population, has yielded 64 bacterial and fungal cultures.

I BIOLOGICAL INVESTIGATION

A. LIMITING FACTORS IN GROWTH RESPONSE

The test organisms and test soils continued to yield good positive responses. However, as data with the laboratory automated monitoring system accumulated, it became evident that the shape of the curve was essentially the same for most of the tests despite differences in the absolute values. The initial responses were rapid, but no further increases of activity were observed after several hours, and the resultant curves reached a plateau (Figure 2). Although the type of response, when compared to the sterile control, generally indicated metabolism; there was no indication of growth. This may result from one or more of several factors. Because most of the responses were observed in the simple salts medium, it is possible that some nutrients might be limiting since an inadequate supply of carbon, nitrogen, vitamins, or minerals could be responsible. Insufficient amounts of available CO_2 might also have limited continued metabolism and growth. It is also possible that the available oxygen became limiting, causing the system to become predominantly anaerobic. A fourth possibility is that the $C^{14}O_2$ collectors became saturated. Of further interest is the observation that this limited growth curve rarely occurs in the field test responses. This suggests that even though the laboratory chambers and monitoring equipment approximate those employed in the field, there may be pertinent differences.

B. RESPONSES TO GLUCOSE CONCENTRATIONS

Efforts to evaluate the concentration of glucose for inclusion in the medium have been intensified. Since glucose serves as an energy source as well as a source of carbon for synthesis, it is important to have a sufficient amount available for heterotrophic growth. The selection of an optimum value is difficult when mixed microbial populations in soils are considered because the concentration must be high

enough to be of value to those organisms capable of utilizing it, but low enough to exclude possible inhibition of other organisms.

The present total concentration of glucose, all uniformly labeled, is 0.005% w/v. The first set of experiments were designed to determine if the 0.005 % was sufficient. To examine the response to various amounts of glucose, successively greater concentrations of cold glucose were added until 1.005 % w/v was present in the medium. The experimental details follow.

Method ----- Automated monitoring and recording system

Medium ----- M9, 0.5 ml

C^{14} Substrates -----	Sp. Act.	mM	uc/ml	% (w/v)
formate	25.00	0.26	6.5	0.002
glucose	4.73	0.28	1.3	0.005
lactate	5.00	0.26	1.3	0.002
glycine	4.42	0.22	1.0	0.002

Inocula ----- Apple Valley soil 100 mg
Rocky Mountain soil 75 mg
Escherichia coli 2×10^6 cells
Saccharomyces cerevisiae 9×10^3 cells

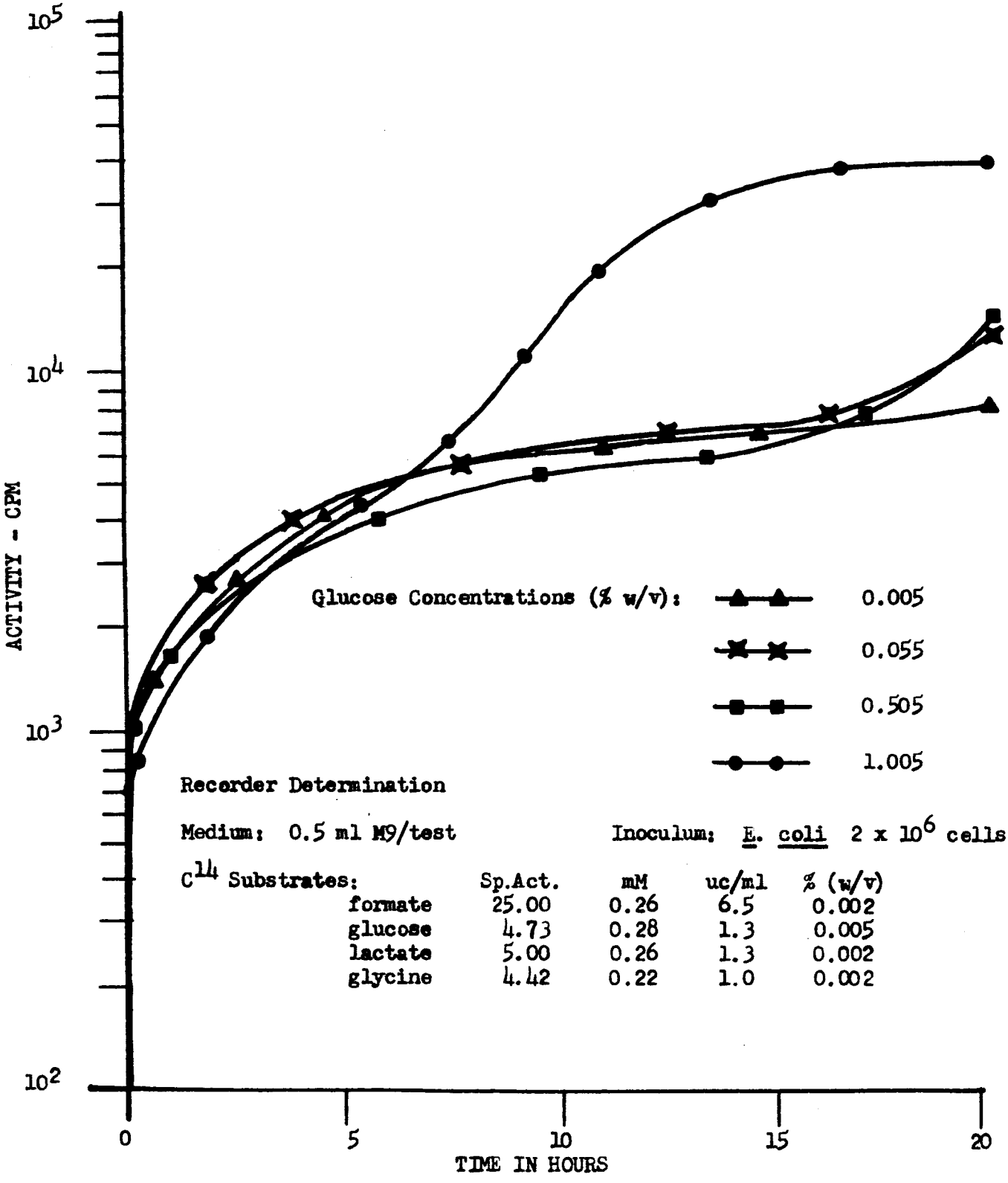
Total incubation and collection period ----- Continuous for 18 hours

Concentration of glucose ----- 0.005, 0.055, 0.505, 1.005 % w/v (0.005% was radioactive in each medium)

Results ----- The medium containing only the standard complement of glucose (0.005%) supported, with one exception, either equal or slightly better responses than the media with higher concentrations. The exception and only definite growth response, was obtained with E. coli (Figure 1). These results indicate that the addition of glucose alone to M9 is not sufficient to increase growth of the microorganisms in the soil. It is probable that glucose supplied enough energy and carbon for the E. coli to synthesize additional needed growth factors, whereas the soil microorganisms were incapable of using the glucose alone for such synthesis. The tests also showed that the CO_2 collectors are capable of picking up additional $C^{14}O_2$ beyond the time at which previous curves reached a plateau. This eliminates getter saturation as the cause of the early plateau in the curves.

Figure I

RESPONSE OF ESCHERICHIA COLI TO CONCENTRATIONS OF GLUCOSE IN M9 MEDIUM



Recorder Determination

Medium: 0.5 ml M9/test

Inoculum: *E. coli* 2 x 10^6 cells

C^{14} Substrates:	Sp.Act.	mM	uc/ml	% (w/v)
formate	25.00	0.26	6.5	0.002
glucose	4.73	0.28	1.3	0.005
lactate	5.00	0.26	1.3	0.002
glycine	4.42	0.22	1.0	0.002

A second set of experiments was conducted to determine if an increased concentration of labeled glucose and a similar concentration of cold glucose would produce observable differences in the $C^{14}O_2$ detected. The standard amount of labeled glucose, 0.005 % was compared with 0.205 % labeled glucose, and with 0.200 % cold glucose (containing, in addition, the standard C^{14} complement of 0.005 %). Experimental details are summarized below.

Method ----- Automated monitoring and recording system

Medium ----- M9, 0.5 ml

C^{14} Substrates -----	Sp. Act.	mM	uc/ml	% (w/v)
formate	25.00	0.26	6.5	0.002
glucose	4.73	0.28	1.3	0.005
lactate	5.00	0.26	1.3	0.002
glycine	4.42	0.22	1.0	0.002

Inocula -----	
Apple valley soil	100 mg
Rocky Mountain soil	50 mg
E. coli	1×10^6 cells
S. cerevisiae	1×10^4 cells

Total incubation and collection time ----- Continuous for 18 hours

Concentration of glucose ----- 0.005, 0.205 % (w/v)

Results ----- It is interesting that all concentrations of glucose, labeled and unlabeled, produced similar responses from both the soil microorganisms and the pure cultures. Metabolism was detected, but definite growth was not apparent. Only two conditions differed between these experiments and the initial experiments in which glucose concentration was evaluated. The first was the maximum concentration of glucose in the second experiment, which was only 20% of the maximum concentration used in the first. The second condition which differed was the history of the inocula. In the first experiment (Figure 1), the inoculum producing the exponential response was grown in tryptone-glucose whereas in the second, the inoculum was grown in Difco Nutrient Broth. It may be that 0.205 % glucose was an insufficient amount even for E. coli or that some constituents in the tryptone-glucose broth affected the response of the E. coli in the first experiment. The latter seems more

probable since a slight response was noticed even at 0.055%. Consequently, an investigation of the effects of constituents in tryptone-glucose broth is now in progress.

C. ADDITIONAL MEDIUM DEVELOPMENT

Throughout most of the year, the basal medium used was M8 (Table 1). This simple medium was designed primarily for the support of organisms in soil. Comparative determinations (Second Annual Progress Report) indicated that dilution or omission of some of the complex constituents of M5 was beneficial. Further study indicated that an inorganic salts medium (M8) plus the radioactive organic substrates produced results equal to, or better than, the diluted M5 from soil inocula. The responses from the M8 have been under continual investigation. Whereas the M5 was believed to contain excessive amounts of organic compounds, the M8 medium might be too limiting. Determinations in which additional nutrients are incorporated in low concentrations are presently in progress. One of these additional nutrients is carbon dioxide. Carbon dioxide is not only a required carbon source for strict autotrophs, but is also necessary for heterotrophic growth. This CO_2 requirement can be replaced by addition to the medium of various organic compounds which are, in themselves, products of CO_2 fixation. As a result, determinations are planned in which the simple salts medium will be supplemented with one or more 4-C dicarboxylic acids such as aspartic, glutamic, succinic, fumaric, and oxaloacetic.

In an earlier study (Second Annual Progress Report) a labeled soil extract medium produced good responses. The possibility that some advantage for soil microorganisms might be obtained by the addition of soil extract to the M8 was indicated. Panmede, a medium supplement supplied by Paines and Byrne, Ltd., was also investigated. This product, a digest of liver, contains vitamins, amino acids, and trace elements. A planchet determination was carried out on two pure

Table 1

COMPOSITION OF BASAL MEDIA

Medium M9	(C^{14} substrates)	Concentration (g/l)
	K_2HPO_4	1.0
	KNO_3	0.5
	$MgSO_4 \cdot 7H_2O$	0.2
	NaCl	0.1
	Soil extract	100.0 ml
Medium M8	(C^{14} substrates)	
	K_2HPO_4	1.0
	KNO_3	0.5
	$MgSO_4 \cdot 7H_2O$	0.2
	NaCl	0.1
Medium M5	(C^{14} substrates)	
	K_2HPO_4	1.0
	KNO_3	0.5
	$MgSO_4 \cdot 7H_2O$	0.2
	NaCl	0.1
	$FeCl_3$	0.01
	Na_2SO_3	0.2
	Malt extract	3.0
	Beef extract	3.0
	Yeast extract	13.0
	Ascorbic acid	0.2
	L-cystine	0.7
	Bacto casamino acid	4.0
	Proteose peptone #3	20.0
	Soil extract	250.0 ml

cultures and three soils. The media compared were:

M8	
M8 plus Panmede	1 g / l
M8 plus soil extract	100 ml / l
M8 plus soil extract and Panmede	100 ml - 1 g / l

All four media contained the formate-glucose-lactate-C^{14} substrates. The results are presented in Table 2. Although dramatic responses were not produced, the addition of soil extract appeared to enhance the response slightly and did not alter the sterile control level. It will be included in the medium in an effort to provide additional supplementary nutrients. The slightly higher sterile controls resulting from the addition of the Panmede, and the lack of increased beneficial results suggested that it not be added to the medium at this time. It may be reconsidered later. The composition of the new medium (M9) is presented in Table 1.

The M9 medium was used in the california field tests. The planchet determinations (Table 4) carried out at that time using M5 and M9 emphasize the difference in responses obtained between the relatively simple medium and a nutritionally rich medium. The M9 produced an approximate tenfold advantage.

Supplementation of M9 with asparagine, ammonium chloride, increased amounts of potassium nitrate, peptone, tryptone, and beef extract is now being evaluated.

Table 2

CARBON DIOXIDE EVOLUTION IN RESPONSE TO GROWTH FACTORS
MEDIA COMPARISON

INOCULUM	INCUBATION PERIODS * hours	NET RADIOACTIVITY - CPM Medium ** M8	M8 Panmede	M8 Soil Extract	M8 Soil Extract Panmede
Apple Valley Soil	0-1	3,086	2,815	3,233	2,792
100 mg	1-2	2,899	2,840	3,220	2,900
	2-4	2,730	3,234	2,867	2,642
Metuchen Soil	0-1	52,742	58,344	66,698	60,650
100 mg	1-2	17,576	33,111	34,910	42,178
	2-4	4,948	3,063	4,370	3,478
Florida Saline Soil	0-1	62	36	21	37
100 mg	1-2	59	C	80	70
	2-4	68	219	172	617
Escherichia coli	0-1	9,279	9,675	9,133	8,982
	1-2	17,871	25,454	21,412	31,287
	2-4	51,166	50,504	50,046	50,400
Bacillus subtilis var globigii	0-1	8,655	12,338	14,308	12,382
	1-2	21,173	41,967	39,730	44,896
	2-4	73,691	24,158	50,235	30,350
Media Controls	0-1	43	125	74	118
	1-2	60	176	56	146
	2-4	49	146	44	141

*Each incubation was followed by a 15 minute $C^{14}O_2$ collection period.

**C^{14} Substrates ----

	Sp.Act	mM	uc/ml	% (w/v)
Formate	25.00	0.26	6.5	0.002
Glucose	4.73	0.28	1.3	0.005
Lactate	5.00	0.26	1.3	0.002

D. NEW SUBSTRATES

In the course of the current program, three radioactive substrates were investigated for possible inclusion in the basal medium. They were DL-α-alanine-1-C^{14}, DL-tyrosine-1-C^{14}, and glycine-1-C^{14} which was investigated previously. The experimental details for the alanine and tyrosine determinations follow.

Method ----- Automated monitoring and recording system

Medium ----- M8 0.5 ml

C^{14} Substrates ----- Alanine determination

	Sp. Act.	mM	uc/ml	% (w/v)
formate	25.00	0.40	10.0	0.003
glucose	4.73	0.40	2.0	0.007
formate	25.00	0.26	6.5	0.002
glucose	4.73	0.28	1.3	0.005
alanine	7.70	0.26	2.0	0.002

Tyrosine determination

formate	25.00	0.40	10.0	0.003
glucose	4.73	0.40	2.0	0.007
formate	25.00	0.26	6.5	0.002
glucose	4.73	0.28	1.3	0.005
tyrosine	3.70	0.26	2.0	0.005

Inocula ----- Alanine determination

Apple Valley soil	100 mg	*E. coli*	8×10^5 cells
Rutgers soil	100 mg	*B. subtilis*	5×10^4 cells
Iron-rich soil (Orange, Va.)	100 mg	var *globigii*	

Tyrosine determination

Metuchen soil	100 mg.	*E. coli*	1×10^6 cells
Iron-rich soil (Orange, Va.)	100 mg	*B. subtilis* v. *globigii* - *S. cerevisiae*	9×10^3 cells

Total incubation and $C^{14}O_2$ collection period --- continuous for 15 hours

Results --- The addition of alanine to the medium did not result in appreciable enhancement in responses from any of the test organisms or soils and will not be considered further. The effect of tyrosine was not very pronounced, but was present in all except the S. cerevisiae determination. The responses were either equal to the formate-glucose standard or slightly better, particularly in the initial hours. Additional determinations are planned on a wider range of test organisms and soils.

Previous investigations (Quarterly Progress Report No.9) of glycine-1-C^{14} indicated that its presence in the medium was beneficial, particularly with soil inocula. In the initial study, glycine was compared with the formate-glucose standard C^{14} substrate in M8 medium. Since then, sodium lactate-C^{14} has been incorporated, and the M8 medium altered and referred to as M9. As a result, determinations were performed using the modified basal medium - M9. The experimental details follow.

Method ---- planchet

Medium ---- M9, 0.5 ml

C^{14} Substrates ----

	Sp. Act.	mM	uc/ml	% (w/v)
formate	25.00	0.24	6.0	0.002
glucose	4.73	0.28	1.3	0.005
lactate	5.00	0.26	1.3	0.002
formate	25.00	0.24	6.0	0.002
glucose	4.73	0.28	1.3	0.005
lactate	5.00	0.26	1.3	0.002
glycine	4.42	0.22	1.0	0.002

Inocula ---- 100 mg
Apple Valley soil
Rocky Mountain soil
Iron-rich soil (Orange, Va.)
California salt soil

Incubation periods ---- 0.5, 1.0, 2.0, 4.0 hours

$C^{14}O_2$ collection time ---- 15 minutes following each incubation period

Results ----- The responses obtained from the substrate containing the glycine were not substantially greater than the standard substrate on these particular soils.

If certain compounds (particularly the Miller compounds) which are considered to be of potential value for Martian microorganisms are found to be compatable with the basal medium, they will be added even though no immediate beneficial effects are apparent with the test organisms. Consequently, the standard C^{14} substrate now includes 0.22 mM, 1.0 uc/ml, and 0.002% w/v of glycine-1-C^{14}.

E. ANTIMETABOLITES

Three compounds were screened for their inhibitory capabilities - mercuric chloride ($HgCl_2$), Argyrol (a commercial preparation of 10 % silver protein), and acrolein (CH_2=CHCHO). The acrolein had been tested previously at a lower concentration. Since this was an initial screening, the inhibitors were not heated to the prescribed sterilization temperature of 135°C for 26 hours. Experimental details of the determination follow.

Method ----- planchet

Medium ----- M8, 0.5 ml plus 0.1 ml of inhibitor

C^{14} Substrates -----

	Sp.Act.	mM	uc/ml	% (w/v)
formate	25.00	0.26	6.5	0.002
glucose	4.73	0.28	1.3	0.005
lactate	5.00	0.26	1.3	0.002

Inocula ----- Iron-rich soil (Orange, Va.) 100 mg
E. coli 0.1 ml of a diluted broth culture

Incubation periods ----- 2.0 and 4.0 hours

$C^{14}O_2$ collection period ----- 15 minutes following each incubation period

Inhibitors ----- final concentration

Mercuric chloride	0.200 %
Argyrol	1.000 %
Acrolein	0.003 %

Results ----- None of the compounds tested proved to be a suitable antimetabolite. The acrolein, which had appeared promising in earlier experiments, produced very high control values in the two hour collection. This was not evident at the lower concentration of 0.002 %. The compound produced partial inhibition of E. coli, but was not effective in the soil. The mercuric chloride reacted with the medium to cause foaming, consequently producing inconsistent values. The Argyrol caused partial inhibition of the soil organisms and of E. coli, but also resulted in high, inconsistent controls.

Although the Bard Parker germicide presently in use continues to be effective, (Figures 3, 5, 7, 9, 10), the inhibitor study will continue until the selection of the most effective antimetabolite is assured.

F. TEST MICROORGANISMS

1. PURE CULTURES

Pure cultures, soil isolates, and soils have been added to the test collection. The known pure cultures with their pertinent characteristics are given below:

(1) Lactobacillus plantarum (ATCC 8014), microaerophilic, heterotrophic, non-motile, gram positive rod, produces little or no carbon dioxide from sugar fermentation.

(2) Clostridium perfringens - strict anaerobe, heterotrophic, spore former, non-motile, gram positive rod.

(3) Aerobacter aerogenes (ATCC 8308), aerobic, facultative anaerobe, heterotrophic, non-motile, gram negative rod.

(4) Sphaerotilus natans * (strains 3, 52), sheathed bacterium, aerobic, heterotrophic, motile, gram negative rod, may or may not be a true iron bacterium.

(5) Leptothrix cholodnii * (strains 1, 19, 99)
Leptothrix discophora (strains 35, 37, 63)
Leptothrix lopholea (strain 76)
Leptothrix pseudo-ochracea (strain 41V), sheathed bacterium, aerobic, heterotrophic, gram negative rod, true iron bacterium.

*Obtained from Dr. E. G. Mulder, Agricultural University, Wageningen, The Nehterlands.

A preliminary determination was performed on the Lactobacillus plantarum to determine if metabolic $C^{14}O_2$ was produced in a measurable quantity from the basal medium. Five tenths ml of M9 medium containing the formate-glucose-lactate-glycine-C^{14} substrate was seeded with 0.1 ml of a direct broth inoculum and incubated in planchets. Fifteen minute wet $Ba(OH)_2$ collections were made following each incubation period. Ninety-three cpm were detected in 0.5 hours; 160 cpm in 1.0 hour; and 151 cpm in 4.0 hours.

Taking into consideration the simplicity of the medium (the Lactobacilli require complex organic media), the results are promising. Future determinations will include the use of nutritionally rich media.

The other bacteria will be tested in the near future.

2. SOILS

Soils continue to be used as inocula, in parallel with pure cultures, in all determinations to insure as complete an evaluation of the basal medium as possible. The soil samples, obtained from various environments (field, forest, sand, rock, iron-rich, saline, etc.) provide a wide range of heterogeneous populations. The recent remote field test environments have been the source of four new soils :

(1) An iron-rich field soil from Orange, Virginia.

(2) Rocks-pebbles, bearing lichens and little free soil, from above the timber line on Sheep Mountain, in the White Mountains, California.

(3) Sand from the Mojave Desert, Death Valley, California.

(4) Saline soil from the Salton Sea flats in California.

In addition, sand from the ocean's edge in Daytona Beach, Florida, has provided a third saline soil for the collection. All the soils tested have given positive responses. Figure 2 depicts the typical curves obtained from the Florida saline soil, the iron-rich soil, and the Metuchen forest soil (described in Progress Report 10). Plate count results given in Table 3 illustrate the wide range of total cell numbers present in the various soils.

3. SOIL ISOLATES

Isolation of colonies from the soils is continuing in an effort to determine possible common sources of the general response obtained from a heterogeneous population.

Five soils have been screened, isolates obtained, and gram stains made. Primarily on the basis of gross morphological characteristics, the following isolates are believed to be different.

(1) Twenty two bacteria, five of which appeared in more than one soil.

(2) Sixteen streptomycetes, one appearing in two of the soils.

(3) Two Nocardia.

(4) One yeast.

(5) One alga.

(6) Twenty two fungi, including two which were isolated from three of the soils, and three which appeared in two of the soils.

Comparative determinations of the responses of the soil isolates are now in progress.

Figure 2

RESPONSES FROM SOILS TO M8 MEDIUM

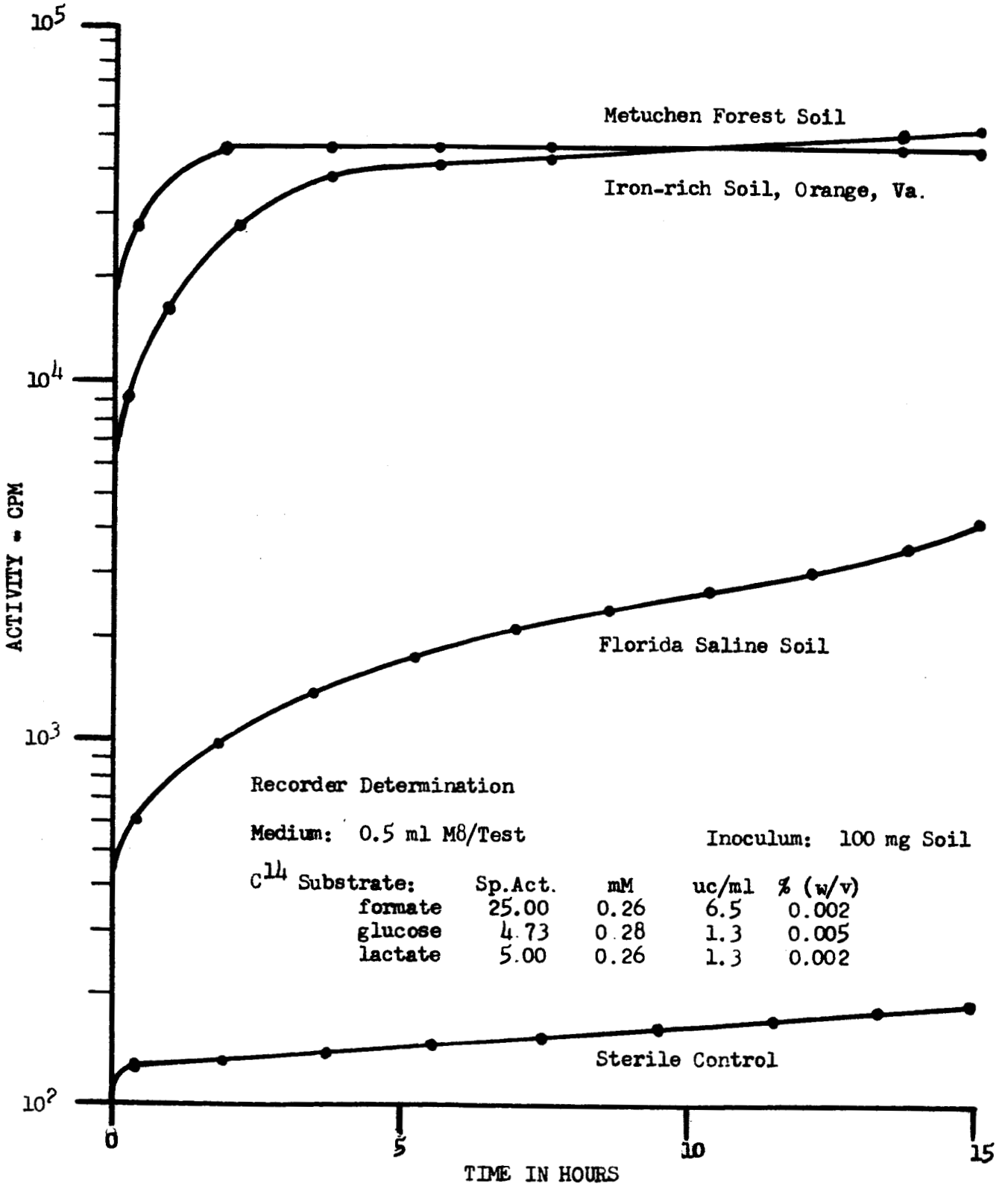


Table 3

COLONY COUNTS OF MICROORGANISMS FROM TEST SOILS

SOIL	LOCATION	COLONIES / 100 mg	
		Tryptone-glucose Agar	Sabouraud Agar
Forest	Metuchen, N.J.	81,000	20,000
Iron-rich	Orange, Va.	148,000 7,500 algae	19,500
Sand	Death Valley,California	1,950 3,950 algae	- *
Rock	Sheep Mountain, California	28,000	- *

* Presently under determination.

II FIELD TESTING IN EXTREME ENVIRONMENTS

Four areas, each having some particularly interesting and severe environmental characteristic, were selected as sites for field testing Gulliver. The first test was conducted on the grounds of the Piedmont Experimental Station of The Virginia Polytechnic Institute at Orange, Virginia. At this particular location the soil contains oxides equivalent to approximately ten percent free iron at the surface. It is a red, hard clay which presents greater difficulty to the Gulliver sampling device than normal dusty or sandy soils. The site was thus a good test of the Gulliver sampling system, and the high iron content was of interest because of the speculation that the redness of Mars results from the presence of iron salts.

Although the test area was freshly plowed, it was quite dry and hard. The pH of the soil was about 6.5. Two instruments were inoculated, and antimetabolite was introduced into one chamber after it had reached approximately 1,000 cpm. The conditions and results of the test are presented in Figure 3. The antimetabolite was effective, although not immediately after being introduced into the chamber. It has been consistently observed that when the antimetabolite is immediately mixed with the medium and diffuses through the retrieval line, it affects metabolism much earlier than when introduced into the chamber after the medium has diffused and metabolism has begun.

The test at Orange was successful. The instruments functioned well mechanically, the soil sample collected was light but adequate, and the difference in response between the inhibited and uninhibited tests was apparent in less than four hours.

The three remaining severe environmental tests were all conducted in California. On October 16, 1963, a test was conducted in the White Mountains of California at a site well above the timber line. The location was Sheep Mountain at an altitude of 12,200 feet. In addition to lack of vegetation, lower atmospheric pressure, and

FIGURE 3

FIELD TEST - GULLIVER III

Date: September 26, 1963 — Weather: 20°C, Sunny

Location: Orange, Virginia — Orientation: Detector up

Ground Condition: Dry, clay — Soil Sample Collected: Light

Medium: 3 ml, M8, 9.1 uc/ml — Antimetabolite: 0.5 ml Bard Parker

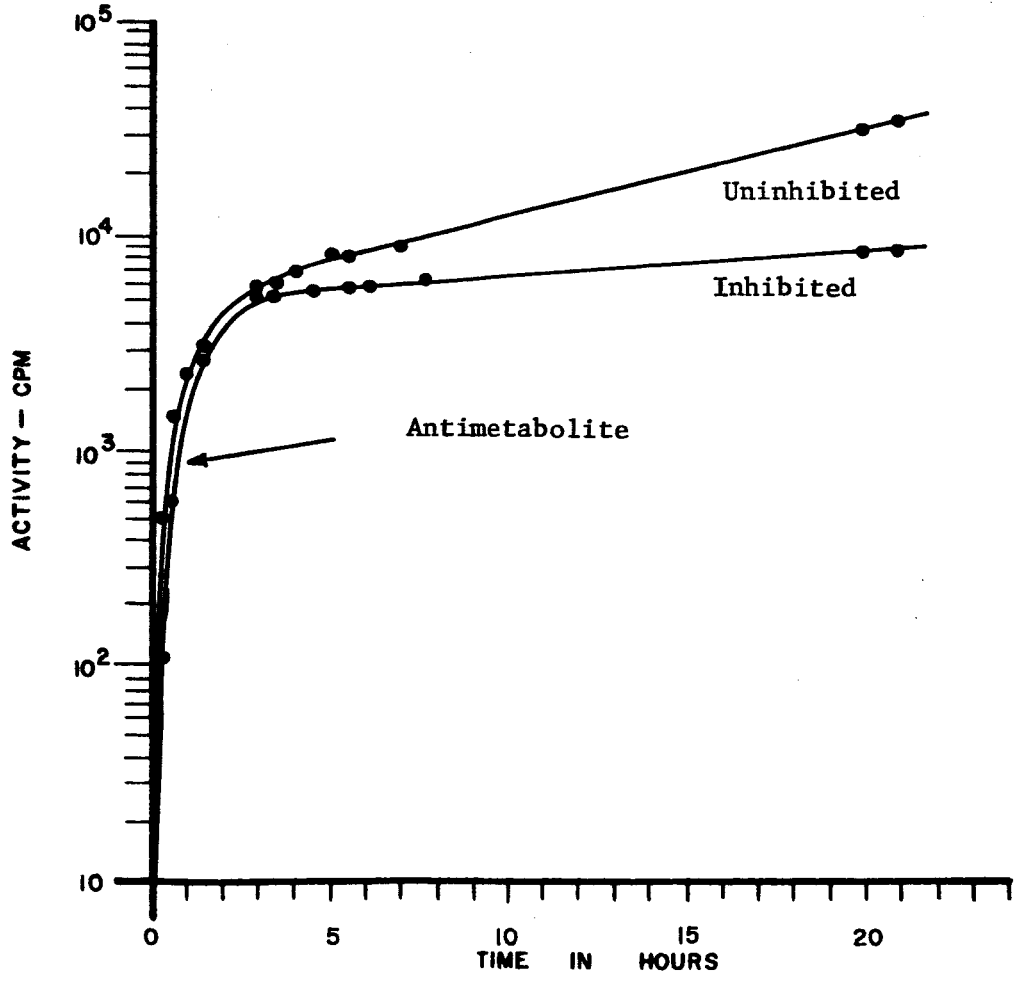
Radioactive Substrates:	Sp. Act. (mc/mM)	uc/ml	mM	%(w/v)
(a) Sodium formate -	25.00	6.5	0.26	0.002
(b) D-glucose U.L. -	4.73	1.3	0.28	0.005
(c) DL-sodium lactate-1 -	5.00	1.3	0.26	0.002

Mechanical Function:
(a) Sequencing: No problems
(b) Projectiles: No problems
(c) Thermostat: Connected

Components:
(a) Detector: Geiger Müller tube
(b) Collector: AMF Tissuglas
LiOH

General Evaluation: Very satisfactory

Response:



relatively cool temperatures, the site selected also provided a difficult test for the soil sampling system of Gulliver. As can be seen in the photographs (Figure 4), the test area consisted mostly of flat, sharply fractured rocks of various sizes. At the time the test was initiated, the weather was clear, cold and windy. However, within minutes after the projectiles were fired, a heavy snow storm started.

One projectile failed to fly free of the retrieval line but the payout of the line was still good. Another line broke as it was dragged over a jagged rock. The unit with the one broken line and one complete line had inhibitor introduced into it immediately. The temperature was -2°C, and, as noted above, snow began to fall shortly after the test was initiated. However, test monitoring was continued until a result was obtained some 50 minutes later. The blizzard stranded the field test crew on the mountain top until the following day. This not only forced the early termination of the test in the field, but prevented continuation of data collection inside the vehicles.

The conditions and results of the test are presented in Figure 5. A distinct difference was readily apparent between the inhibited control unit and the uninhibited test unit as early as 20 minutes after the test started. After 50 minutes, the uninhibited chamber read 1,782 cpm and the inhibited chamber read 520 cpm, a three-fold difference. With the exception of the failure of one projectile to free itself completely and one retrieval line breaking, the mechanical operation was satisfactory. In spite of the adverse environmental conditions, the test was felt to be highly successful within the 50 minute period.

The next test was conducted in Death Valley between Baker and Shoshone. The test was performed on a shifting sand dune (Figure 6). The weather was generally fair, the air temperature was 29°C and the sand temperature was 32°C. There was a seven m.p.h. wind. The fact

Figure 4

FIELD TEST - SHEEP MOUNTAIN, WHITE MOUNTAINS, CALIFORNIA

The testing site, illustrating the adverse environmental conditions, notably the barren rocky terrain and cold temperatures.

FIGURE 5

FIELD TEST - GULLIVER III

Date: October 16, 1963 — Weather: -2°C, windy

Location: Sheep Mountain, White Mountains, California — Orientation: Detector up

Ground Condition: Mainly rock, little soil — Soil Sample Collected: Light

Medium: 3 ml, M9, 9.66 uc/ml — Antimetabolite: 0.5 ml Bard Parker

Radioactive Substrates:	Sp. Act.(mc/mM)	uc/ml	mM	%(w/v)
(a) Sodium formate -	25.00	6.0	0.24	.002
(b) D-glucose-U.L. -	4.73	1.3	0.28	.005
(c) DL-sodium lactate-1 -	5.00	1.3	0.26	.002
(d) Glycine-1 -	4.42	1.0	0.22	.002

Mechanical Function:
(a) Sequencing: No problems
(b) Projectiles: One projectile remained attached
(c) Thermostat: Functioning

Components:
(a) Detector: Geiger Müller tube
(b) Collector: AMF Tissuglas LiOH

General Evaluation: Overall test was satisfactory. An unexpected blizzard forced an early termination of the test.

Response:

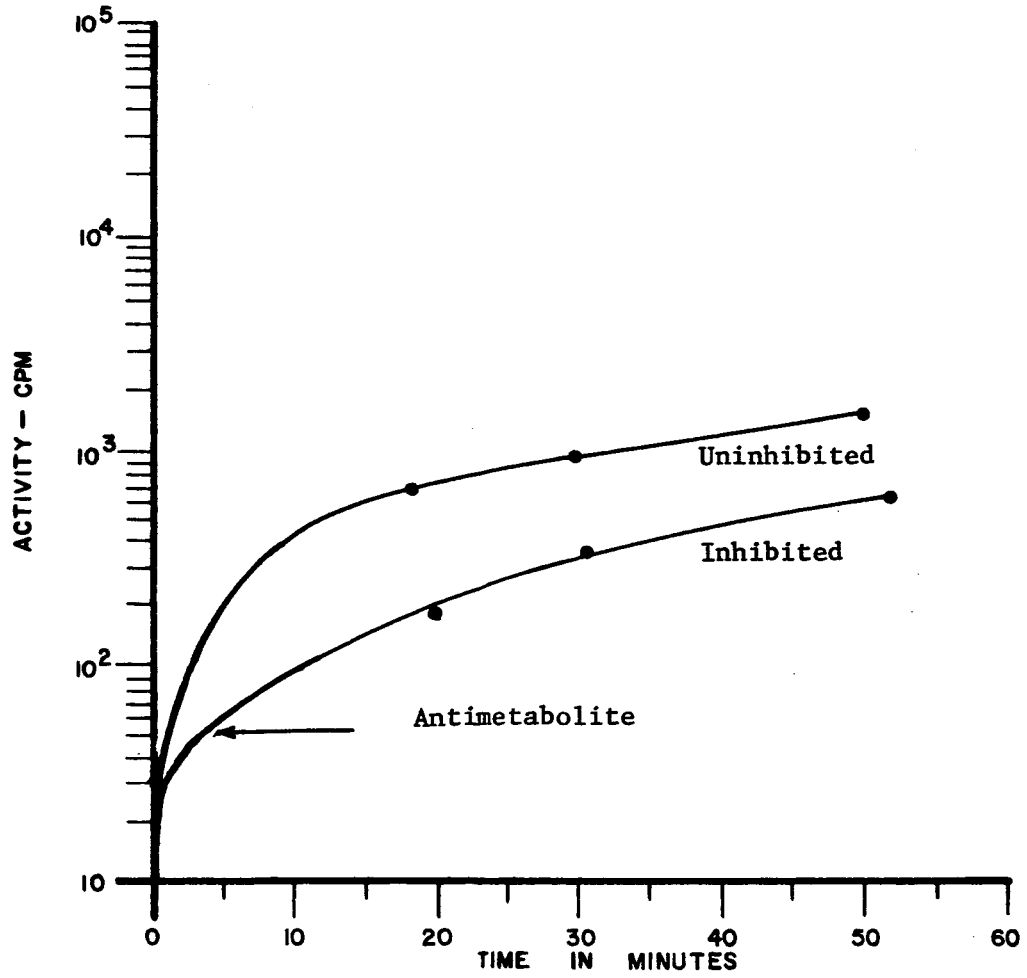
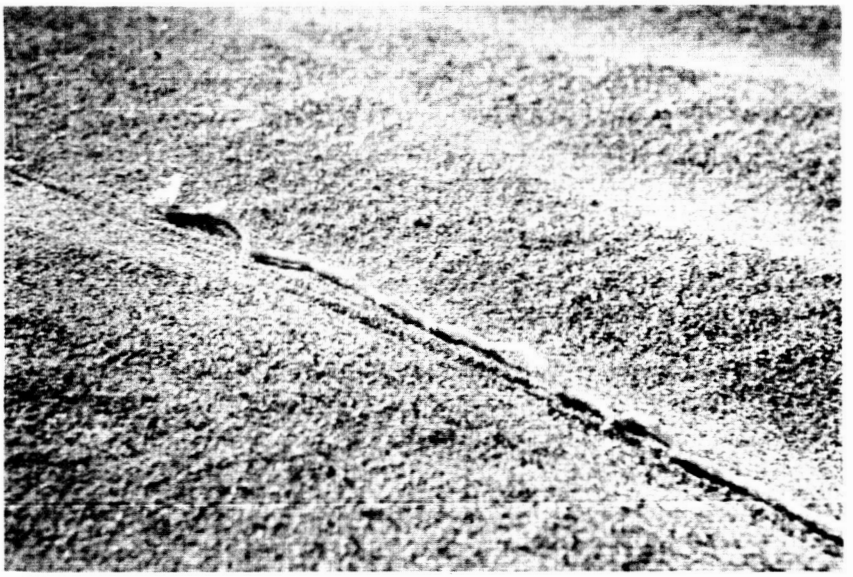


Figure 6

FIELD TEST - DEATH VALLEY, CALIFORNIA

Testing site. Paths left by collecting lines are clearly visible.



Close-up of the chenille portion of the collection line passing over sand grains. The sand can be seen adhering to the line.

that the sand consisted of rather large grains was a further test of the Gulliver sampling method. In addition, the area is arid which may influence the microbial soil population in a manner similar to the influence of the much more arid Martian conditions. Although there were abnormal showers in the area several days prior to the test, the surface soil was dry at the time of the test. The siliconized lines collected a very heavy soil sample. One projectile of each test unit failed to carry free of the lines, an event which proved to be helpful in that the additional weight on the line increased the amount of soil picked up. After some two and one half hours of *in situ* monitoring, rather than terminate the test because of darkness, an attempt was made to transport the units to the vehicles for continued monitoring. In the moving, the antimetabolite ampoule in the test unit was inadvertently broken, resulting in inhibition of that unit as well as the control unit which had been inhibited immediately after the soil sample was introduced into the chamber. The exact time has not been ascertained, but it was probably between two and one half and three hours after the beginning of the test. In Figure 7, the conditions and results of the test are shown. Here again, the effectiveness of the antimetabolite when introduced immediately is apparent. The overall test was highly successful.

The third test in California was conducted on the Salton Sea desert flats of the Imperial Valley (Figure 8). The soil was hard and granular, and evidence of the high salt content could be seen on the surface in the form of crystalline encrustations. Because of the rain mentioned previously, the soil was not as dry as would have been preferred, and showed evidence of moisture in depressions. However, the test area was selected for its dry appearance. Again, one retrieval line broke. In an effort to impose extreme conditions on the test, the unit with only the single line recovered was used as the uninhibited test and the unit with both retrieval lines recovered was inhibited. As can be seen in

FIGURE 7

FIELD TEST - GULLIVER III

Date: October 20, 1963 Weather: 29°C, hot, sunny

Location: Death Valley, California Orientation: Detector up

Ground Condition: Dry, sand dunes Soil Sample Collected: Heavy

Medium: 3 ml, M9, 9.66 uc/ml Antimetabolite: 0.5 ml Bard Parker

Radioactive Substrates:	Sp. Act.(mc/mM)	uc/ml	mM	%(w/v)
(a) Sodium formate -	25.00	6.0	0.24	.002
(b) D-glucose-U.L. -	4.73	1.3	0.28	.005
(c) DL-sodium lactate-1-	5.00	1.3	0.26	.002
(d) Glycine-1 -	4.42	1.0	0.22	.002

Mechanical Function:
(a) Sequencing: No problems
(b) Projectiles: Projectiles remained attached
(c) Thermostat: Connected

Components:
(a) Detector: Geiger Müller tube
(b) Collector: AMF Tissuglas LiOH

General Evaluation: Overall test was satisfactory. Projectiles failed to carry free in both units, but actually increased the amount of sample by adding weight to the lines. Antimetabolite was inadvertently introduced into the test unit sometime between 2.5 hours and 3.5 hours.

Response:

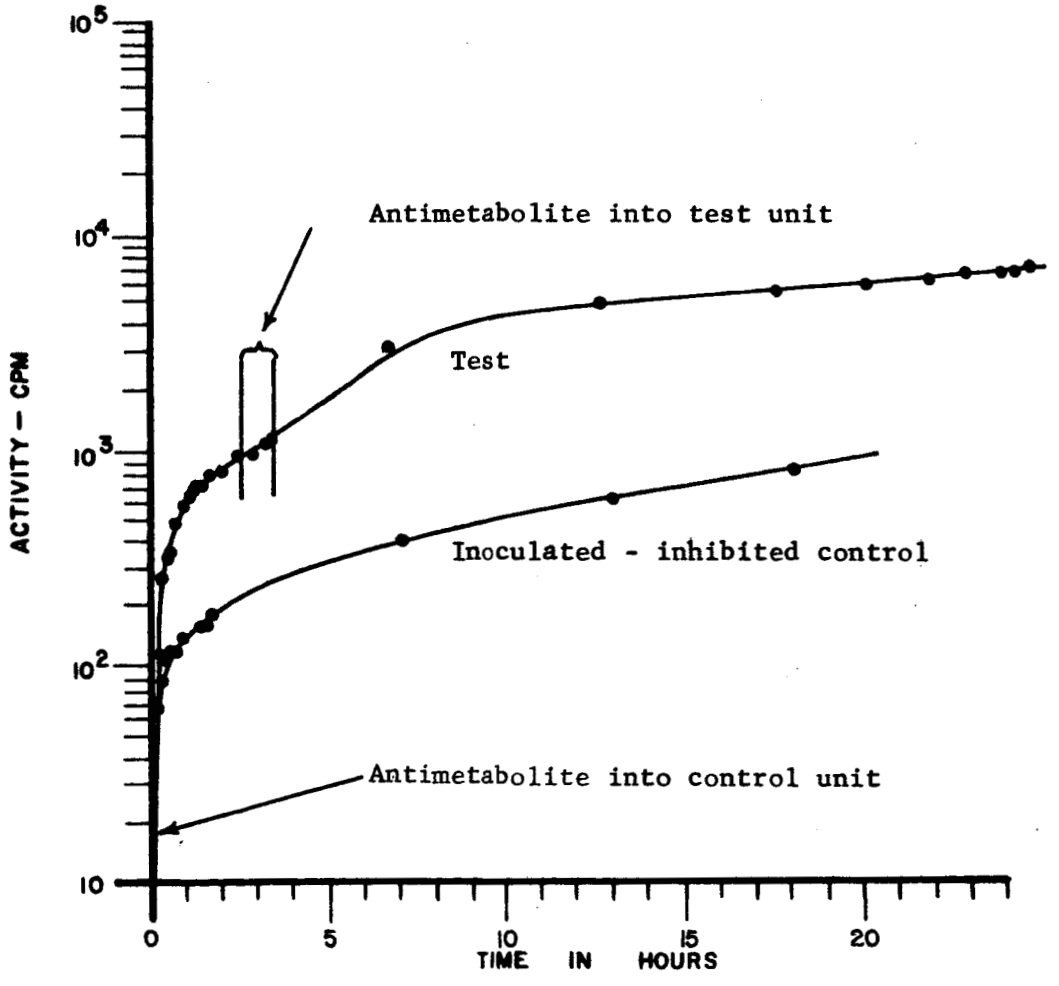
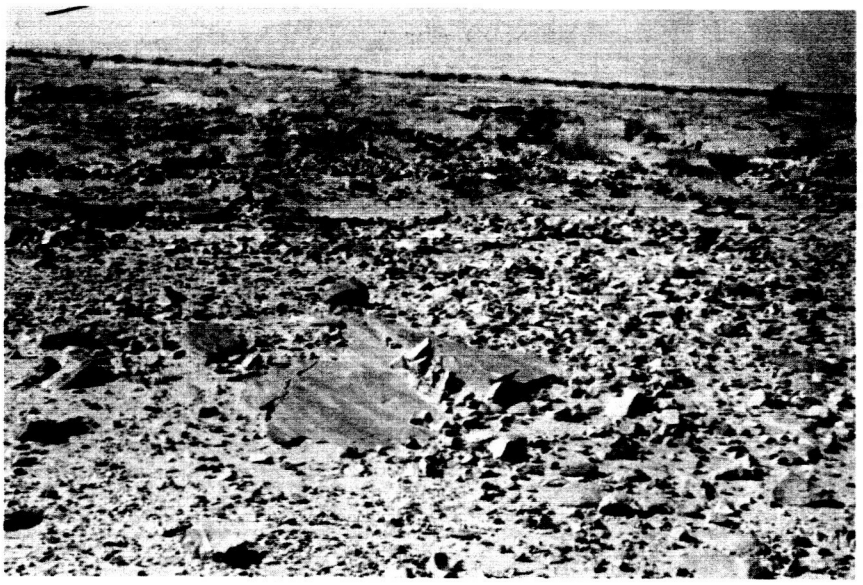


Figure 8

FIELD TEST - SALTON SEA, CALIFORNIA



Testing site, depicting a close up and overall view of the terrain.

Figures 9 and 10, the response was definitive and the test was successful.

Soil samples were collected aseptically at each test area and tested by the planchet method in the laboratory. Approximately 50 to 100 mg of soil were placed into replicate planchets to which 0.25 ml of the complex medium, M5, and the simple medium M9, were added to respective sets. The results are presented in Table 4. They substantiate the field test results, but again, it was demonstrated that the mechanical Gulliver units are much less sensitive than the planchet method. The consistent difference in response between the complex and simple medium in both methods emphasizes the importance of the medium development aspect of the Gulliver program.

The possibility of modifying the Gulliver in a manner which would essentially permit determination of the presence of microorganisms <u>in situ</u> has frequently been discussed by the investigators. In an effort to examine the possibility of utilizing this approach, 0.25 ml of M9 medium was placed directly on soil or rock at the test site in Death Valley and one to two drops at the Salton Sea test site. Planchets containing pads moistened with wet $Ba(OH)_2$ were placed directly over the test area to collect the $C^{14}O_2$ evolved. The results are presented with the planchet data in Table 4. Sterilized soil in M9 medium did not result in activity above the sterile control level. The rapid response from organisms <u>in situ</u> suggests an area for consideration in the future Gulliver program.

In general, the remote field tests, which provided rigorous, terrestrial environmental conditions for the Gulliver instruments, showed the effectiveness of the soil sample collection system, the inhibitory effectiveness of the antimetabolite, and the rapidity of responses obtainable. They were considered to be highly successful and exceptionally instructive in illustrating areas in which improvements should be considered.

FIGURE 9

FIELD TEST - GULLIVER III

Date: October 25, 1963

Weather: 32°C, recent rain

Location: Salton Sea, California

Orientation: Detector up

Ground condition: Hard, granular

Soil Sample Collected: Light

Medium: 3 ml, M9, 9.66 uc/ml

Antimetabolite: 0.5 ml Bard Parker

Radioactive Substrates:

	Sp. Act.(mc/mM)	uc/ml	mM	%(w/v)
(a) Sodium formate -	25.00	6.0	0.24	0.002
(b) D-glucose-U.L. -	4.73	1.3	0.28	0.005
(c) DL-sodium lactate-1 -	5.00	1.3	0.26	0.002
(d) Glycine-1 -	4.42	1.0	0.22	0.002

Mechanical Function:

(a) Sequencing: No problems

(b) Projectiles: One retrieval line broke

(c) Thermostat: Connected

Components:

(a) Detector: Geiger Müller tube

(b) Collector: Collector: AMF Tissuglas LiCl

General Evaluation: Overall test was satisfactory. One retrieval line broke. Uninhibited test conducted in chamber with single line.

Response:

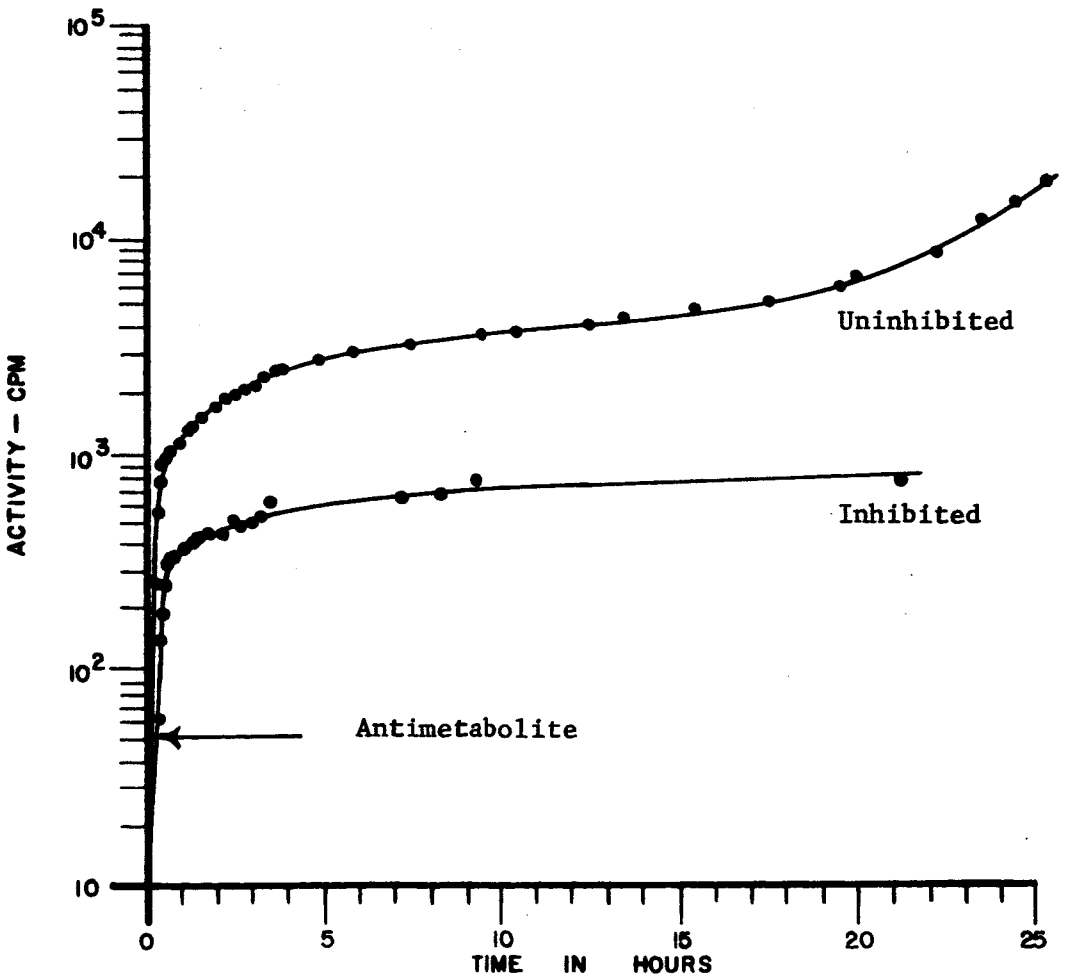


FIGURE 10

SALTON SEA, CALIFORNIA FIELD TEST

RESPONSE- FIRST FIVE HOURS

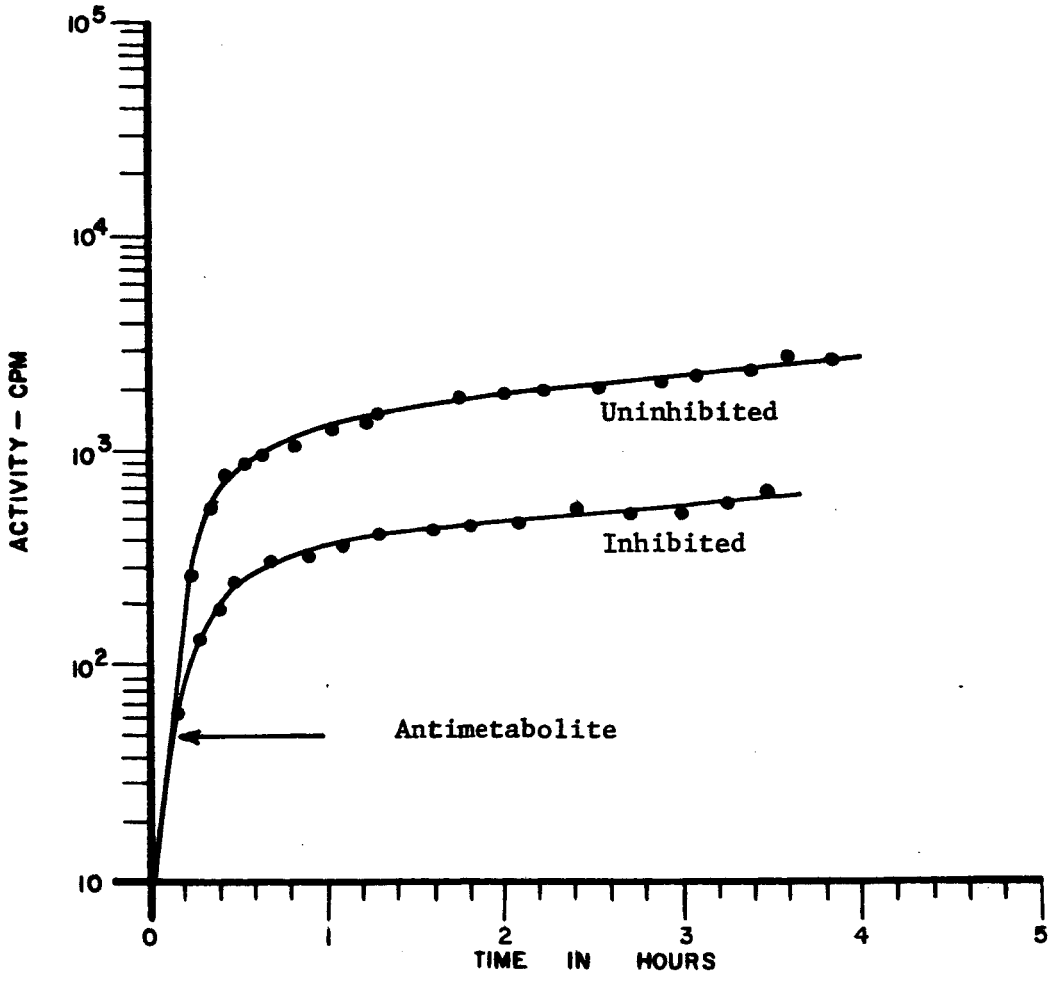


Table 4

RESPONSE FROM CALIFORNIA FIELD TEST SOILS
PLANCHET DETERMINATION

Soil	Medium 0.25 ml	Net Radioactivity - CPM Time Elapsed After Incubation	
WHITE MOUNTAINS		0 to 1.25 Hours	1.25 to 4 Hours
Sheep Mountain) Soil 50-100 mg)	M5	4,389	3,240
Pebbles)	M5	4,229	3,177
50-100 mg)		53,864	30,413
SALTON SEA		0 to 1 Hour	1 to 2 Hours
Soil)	M5	2,156	1,712
50-100mg)	M9	22,439	22,947
		0 to 0.5 Hour	
Direct rock	M9 1 drop	1,980	
		0 to 1 Hour	
Direct Soil	M9 1 drop	40,000	
DEATH VALLEY		0 to 0.75 Hour	0.75 to 1.5 Hours
Soil app. 75 mg)	M5	12	22
Depth - 0.25)	M9	441	605
Depth - 3.00 in.	M5	34	42
	M9	370	555
		0 to 1 Hour	
Direct soil	M9	40,000	

The investigators wish to acknowledge with thanks the generous help provided by Mr. J. B. Carter and Mr. C. J. Koch at Orange, Virginia and the Virginia Polytechnic Institute for granting permission to test on their land.

They also wish to acknowledge the generous aid and participation in the testing program of Dr. Gerald Soffen and Dr. Roy Cameron of the Jet Propulsion Laboratory who provided assistance, vehicles, equipment, and knowledge of the California areas selected for test sites. Without them the task would have been far more difficult.

In addition, the use of laboratory facilities and equipment of the Biology Division of the California Institute of Technology which served as a base of operations, and the facilities of the Crooked Creek Laboratory of the University of California White Mountain Research Station is gratefully acknowledged.

PERSONNEL, MANAGEMENT

1. PERSONNEL

Dr. John M. Barnes, plant pathologist, has become associated with the Radioisotopic Biochemical Probe for Extra-terrestrial Life. Dr. Barnes will be concerned primarily with the photosynthetic aspects of the program.

2. MANAGEMENT

On August 1, 1963 Resources Research, Incorporated, Washington, D.C., became a wholly owned subsidiary of Hazleton Laboratories, Incorporated, a private research and development firm in Falls Church, Virginia. In addition to the present project staff, which will remain unchanged, the merger has made available the services of a larger scientific staff, extensive facilities, and additional equipment.

Quarterly Progress Report No. 11

NASr-10

Respectfully submitted,

Gilbert V. Levin

Gilbert V. Levin, Ph.D.
Experimenter

Norman H Horowitz

Norman H. Horowitz, Ph.D.
Experimenter

Allen H. Heim

Allen H. Heim, Ph.D.
Senior Microbiologist

Mary-Frances Thompson

Mary-Frances Thompson
Biologist

PART III

INSTRUMENTATION

A. Wendell Carriker
Project Manager

Dr. A. A. Sterk, Mgr.
Space Instrumentation
Department

AMERICAN MACHINE & FOUNDRY COMPANY
RESEARCH & DEVELOPMENT DIVISION
1025 NORTH ROYAL STREET
ALEXANDRIA, VIRGINIA

III. INSTRUMENTATION

A. GENERAL

During this reporting period, the principal efforts have been field testing, improving gas collectors, and evaluation of radiation detectors. As mentioned in the previous quarterly report, the CO_2 gas collectors that were being used for field testing as well as for collection of CO_2 in the evaluation of radiation detectors, did not have good reproducibility. This lack of reproducibility existed with gas collectors within a given batch and between different batches. The efforts to improve and evaluate this reproducibility are described in the following section.

There were four field tests performed during the reporting period. All four of these tests were made outside the greater Washington area at sites selected by Resources Research, Incorporated, on the basis of special soil and climate conditions. Categorically, it can be said that all four tests were successful, even though more difficulties were encountered in these four tests than had been encountered in the tests of the preceding two quarters of the program. For these field tests, a third unit was added to the testing set-up. The purpose of the third unit is to serve as an uninoculated sterile control. This third unit is a mock-up which has the same internal configuration and $C^{14}O_2$ collection and detection systems as the test units, but the mechanical operations are actuated manually. However, no means for flushing out nonmetabolic $C^{14}O_2$ were provided.

A switching system was fabricated for sequentially switching the output of the radiation detector of the three test units into the RRI-procured rate meter and chart recorder. This addition to the field test equipment greatly improves the ease with which the field test data can be taken continuously during hours outside the normal working day.

In the area of detector development, the efforts have been focused on experimentally determining the increases in system sensitivity which can be attained by utilizing either a windowless proportional counter or a thin-window counter which would have a geometry approaching 4π.

B. GAS COLLECTION

The last quarterly report included a discussion of development of collectors using the Tissuglas pads as a hydroxide carrier. Investigations of reproducibility of these pads with lithium hydroxide were under way at the time the report was written and are reported here.

1. Process for Making Pads

The technique developed to improve CO_2 collector reproducibility is as follows: A sheet of glass tissue about 1 mg/cm^2 thick is laid on a 1/4" x 4" x 6" teflon board and covered by a stainless steel mesh previously coated with a non-wettable silicone release agent. This stack is then dipped into the hydroxide solution. To help prevent pooling of the solution and resulting nonuniform deposition during drying, a framework with

runners is placed over the wire mesh and secured with "C" clamps to exert a gentle uniform pressure over the entire area of the mesh. This squeezing procedure also prevents the formation of long crystallization dendrites since the mesh presses into the wet tissue. After the excess hydroxide solution is drained off, the assembly is placed level in a vacuum dessicator containing ascarite for drying. When it is dry, the sheet of Tissuglas and hydroxide is cut into pads of the desired dimension using appropriate tools such as scissors or cork cutters.

2. Uniformity of Deposit

Checking for uniformity of LiOH on the Tissuglas was done by cutting small disks from a sheet prepared as above, noting the locations of the disks, and weighing. Analysis of the disk weights from a sheet cut in this manner showed that there is a uniform variation from the edges toward the center, the central portion being lighter. Though this is not the desired over-all uniformity, the technique at least does furnish pads which are quite similar. The weights of pads cut from adjacent areas are typically within 5 to 10 percent. Appearance to the eye is strikingly uniform.

3. Uniformity for Gas Collection

Having found a means of depositing hydroxide fairly evenly on the Tissuglas, a gas collection test was made using chemical generation of

$C^{14}O_2$ and window planchets. Counting was with a geiger tube. The results are given in Table III-1. These data indicate that there is reasonably good uniformity among collectors from a given batch for collection of CO_2.

4. Effect of Atmospheric CO_2

An unresolved problem which has been of concern since the pad technique was developed has been the magnitude of the effect atmospheric CO_2 has on the exposed hydroxide. What contamination or depletion of available OH ions occurs while the pads are being fabricated and stored prior to use? What contribution toward lessened sensitivity comes from allowing the assembled geiger tube and pad to be exposed to air? Tests have indicated that storage in sealed CO_2-free containers has no significant effect on sensitivity.

In order to observe the effect of atmospheric CO_2 on sensitivity after the pads are fabricated, it was necessary to perform an experiment in which the pads were made in an entirely CO_2-free atmosphere. A drybox was used to make the pads for this experiment. It was purged with nitrogen and this gas was recirculated for a day through a CO_2 absorbent trap in the drybox. An unopened bottle of lithium hydroxide was used to prepare the solution in the drybox. Forty pads were made in the manner described above. A set of ten pads was used in each of four tests where the exposure to atmospheric atmospheric CO_2 was 0.0, 0.5, 1.5,

TABLE III-1

$C^{14}O_2$ Collection Test for Reproducibility

Weight of LiOH (mg)	Counts Per Minute	cpm Deviation from Average	cpm/mg	cpm/mg Deviation from Average
24.6	4574	-23.4%	186	-15.8%
24.8	4750	-20.3%	192	-13.1%
26.0	6043	+1.4%	232	+5.0%
26.0	6053	+1.6%	233	+5.4%
26.2	5046	-15.3%	193	-12.7%
26.6	6774	+13.7%	254	+14.9%
27.6	5991	+0.5%	217	-1.8%
28.0	7830	+31.4%	280	+26.6%
28.5	5849	-1.9%	205	-7.2%
29.2	5309	-9.5%	182	-17.6%
29.6	7407	+24.3%	250	+13.1%
av = 27.9	av = 5960	av = 12.1%	av = 221	av = 12.6%

and 5.0 hours before exposure to a constant amount of $C^{14}O_2$. (The pads of the set for the test with no exposure to atmospheric CO_2 were not weighed because it was not possible to fit a proper balance inside the drybox). These ten were made into window planchets and installed and sealed in the test apparatus before removing from the drybox. The apparatus was placed in the test chamber where the constant amount of $C^{14}O_2$ was generated. The remaining 30 pads were sealed in air-tight containers for later weighing and exposure for selected periods of time to the atmosphere. These were then subjected to the same amount of chemically generated $C^{14}O_2$ as the ten which had never been exposed to atmospheric CO_2.

The weight of these pads made in the drybox were not as uniform as those made in unrestricted working conditions, but since each run included ten pads it seems likely that the average result from each run would reveal drastic effects, if any exist for exposure to the atmosphere for periods of several hours. These averages and the time of exposure to the air are given in Table III-2. From these data, no drastic effect on sensitivity is apparent even after exposure to air for five hours. Even though there is more spread in these data than would be desired and it would be advantageous to repeat the tests, it seems safe to conclude that the short periods of exposure to atmosphere such as are necessary in affixing the pads on geiger tubes prior to testing are not harmful to

TABLE III-2

Period of Atmospheric Exposure	cpm Average	cpm/mg Average
0	6,686	Not Weighed
30 minutes	14,010	743
1 hr., 30 min.	17,220	1,252
5 hours	8,380	720

sensitivity. The cpm/mg sensitivity figures for these tests are about a factor of three higher than for sets of collectors fabricated in normal atmospheric air. Repeating the tests should confirm or refute the reality of this apparent effect of atmospheric CO_2 on collector sensitivity during fabrication of the collector pads.

C. DETECTORS

In the previous report, data were reported describing a possible increase in sensitivity of a factor of greater than ten which might be obtainable with windowless flow counters. An attempt was made in those tests to determine the effects of gas collector areas on the windowless detector response to chemically and metabolically generated $C^{14}O_2$. It has been noted in previous reports that water vapor was degrading to the response of the windowless counters, and for the mentioned tests great efforts were expended to prevent water vapor from getting into the detector. The results in that report indicated that windowless counters might increase sensitivity by factors greater than ten. The results of those tests were subject to some questions in that the gas collectors utilized were not very reproducible.

There appear to be two techniques whereby a windowless counter might be utilized for this experiment. In one case, the detector would be separated from the incubation chamber by some type of water vapor trap,

which would permit the metabolically evolved gases to diffuse into the sensitive region of the detector. This would require that the detector and incubation chamber be filled with the counting gas. Exposure of the microbial culture to a counting gas such as argon and methane or helium and butane has not been deemed advisable. Or, the detector would have to be operated with ambient atmospheric gases. With the composition and pressure of the Martian atmosphere not well known, attempting to design a detector with gas amplification (Geiger or proportional) would be nearly impossible. It might be feasible to design a detector to operate as an ionization chamber without gas amplification; however, the anticipated reduced pressure of Martian atmosphere would require that the ion chamber be exceedingly large in order to collect sufficient ionization energy in the chamber to detect significant currents for the quantities or specific activities of the C^{14} gases anticipated.

The second approach to a windowless counter would be a system whereby the incubation chamber and detector are separated by a valve which could be alternately opened and closed. The incubation chamber would always contain Martian atmospheric gases and thereby permit any microbes to grow in their natural gaseous environment. To collect the metabolic gases, the detector would be flushed with Martian gases and the valve to the incubation chamber opened, thereby permitting the metabolically evolved gases to diffuse into the detector where they would be taken up

by appropriate getters (gas collectors). After a short period of time, the valve would be closed and an additional time would elapse permitting the gases to be collected. The detector would then be flushed with an appropriate counting gas and the collected C^{14} tagged gases would be monitored. The detector would then be flushed with ambient Martian atmospheric gases to complete the detection cycle. The cycle would start again with the opening of the valve between the detector and incubation chamber. To test the increase in efficiency which could be obtained with a detection system of the latter type, two devices were fabricated with copper tubing and valves which permitted mounting a windowless detector on one unit and a thin-window geiger tube on the other unit. This testing arrangement differed from those previously described in that the two detectors with their gas collectors (internal for the flow counter and on the window of the geiger) were not competing for the single source of $C^{14}O_2$. In this set-up, identical microbial inoculae were used in the C^{14} tagged broth as a source of gas for each unit. The tests performed indicated that the increase in sensitivity which could be obtained for this windowless detector ranged between a factor of four and six, provided the single gas collector on the geiger tube did not become saturated.

More tests of this type should be performed since only three were run and the results of only two appeared valid.

Another approach to improving detection efficiency is to utilize a window-type detector (proportional or geiger) with a window area larger than that of the currently used Amperex 18515 geiger tube. Several techniques might be used to effect this larger area. These would be:

1. A larger diameter end-window tube.
2. A cylindrical geiger tube with a single central anode, the outer wall (cathode of the tube) being less than 1 mg/cm^2 thick and supported structurally by an appropriate cylindrical grid.
3. A cylindrical detector with concentric walls (cathode) with a multiplicity of anodes running parallel to the axis of the cylinder. The smaller of the two cylindrical walls of this detector would be less than one milligram per square centimeter thick and would be appropriately supported by a structural grid.

With the first two of these schemes, the maximum counting efficiency would approach 50 percent or 2π for a gas collector mounted on the thin window, if one assumes no absorption of beta particles in the collector or the window. With the third system, if the length of the cylinder is large with respect to the diameter of the inner cylinder (window), the detecting efficiency would approach 100 percent or 4π, again, if there were no absorption effects.

To determine the increase in sensitivity which could be attained using a larger window-type detector (either of the cylindrical-window or end-window type) and to determine the effects of gas collector area on system response, a tiny plenum chamber has been fabricated which mounts directly on top of a mock-up of the Gulliver instrument. This mock-up has the same intermal dimensions as the actual instrument and there is provision for generating $C^{14}O_2$ gas chemically or metabolically. The tiny plenum chamber has four ports on the walls which will accommodate thin-window geigers. If the entire wall surface were the windows of geiger tubes, the configuration would approach the detector of case 3 described above. With data taken from the four detectors, calculations will indicate the results that would be obtained for a detector like case 3. Since water vapor will not interfere with the performance of the thin-window geiger tubes, no special precautions need be taken to eliminate water vapor. With this apparatus, tests will be run in which gas collectors will be mounted on one through all four detectors. To determine the effect of gas collector area on sensitivity for a given quantity of gas, the counts from the two, three or four detectors can be added together and compared to the response observed with a gas collector on just one detector. To determine the effect of counting CO_2 in the gas phse, a plate can be inserted diagonally across the chamber so that a bare detector on one side of the chamber will not detect the activity on a collector on the face of the detector on the other side of the chamber.

Tests with this apparatus will be conducted early in the next quarter and reported in the next report.

D. NONMETABOLIC GAS REMOVAL AND SOIL SAMPLE COLLECTION

No experiments were performed in these areas during this reporting period.

E. FIELD TESTS

The response curves of the four out-of-town field tests were shown in the previous section of this report, and the reader is referred to these curves for results. The discussions in this section will be restricted to operational details.

1. Orange, Virginia

The first out-of-town field test (September 26, 1963, at Orange, Virginia) was the first time the automatic recording system was used in the field. Mechanically and electronically, everything operated without difficulty. The amount of soil collected on the lines was not as great as would have been desired; this pointed out that extra care should be taken to put sufficient silicon on the lines to be used for the tests forthcoming in California.

Even though the uninoculated sterile control unit was not flushed, the count rate from this unit was not very high. The inhibited unit received the antimetabolite about twenty minutes after the inoculation was made. The motor for rotating the spool to distribute the antimetabolite out into the lines was not actuated until about three minutes after the antimetabolite ampoule was broken. It is speculated that this resulted in the antimetabolite

not being quickly distributed throughout the inoculum, which, along with the 20-minute incubation of the organisms, resulted in the level of the inhibited unit rising significantly above that of the uninoculated sterile unit.

After approximately 22 hours of operation, the test unit showed a count rate of approximately 42,000 counts per minute. It was decided that the gas collectors on the two inoculated units should be changed to preclude the possibility of losing data because of saturation of the gas collectors by atmospheric CO_2 diffusing into the chamber through the small opening provided for the chamber to "breathe". To check for saturation, the collector pads were removed from the detectors and exposed to chemically generated $C^{14}O_2$ in a small closed container. The count rate of pads was checked before and after this exposure. It was determined that neither of the pads was saturated, but the affinity of the collector from the test unit for CO_2 was less than that of the collector from the inhibited unit. New sets of gas collectors were installed on the two test units within 20 minutes of removal of the first sets, which prevented loss of any significant amount of metabolic CO_2. The count rate from the test unit exceeded 500,000 counts per minute (with corrections made for detector dead time) approximately 30 hours after inoculation (eight hours after installation of the second collector).

2. California Tests

For the three field tests performed in California during October, the majority of the equipment was that used in the local field tests; however,

some of the heavy equipment (such as batteries) was provided by Jet Propulsion Laboratory and the California Institute of Technology. The assembly, autoclaving, and checkout of the system prior to each field test was done in the laboratory of Dr. Norman Horowitz at California Institute of Technology. The dis-assembly and clean-up following each field test was also done in his laboratory. It should be stated that the participation of Dr. Horowitz and Dr. Gerald Soffen and Dr. Roy Cameron of Jet Propulsion Laboratory was greatly appreciated and was a significant factor in these tests being successful.

a. Sheep's Mountain, California
(White Mountain of Inyo National Forest)

This test was started about 6:30 p.m. October 16, 1963. For this test the assembly of the units was completed the evening before. During the trip to White Mountain, the instruments were subjected to rather severe shock and vibration conditions in the back of the carry-all vehicle. At the time the test was started, the outside temperature was a few degrees below freezing, and it started snowing a few minutes after the sample collection lines were retrieved. Since dark was rapidly approaching, the check-out prior to initiating the test was minimized. The two automatic units operated satisfactorily; however, difficulties were encountered with the projectiles. When the first unit was fired, the line paid out of only one projectile completely during the trajectory in air. In the case of the other projectile, part

of the chenille hung up within the projectile but pulled out as the rotary solenoid pulled the line back into the instrument. When the second unit was fired, the lines hung up in both projectiles. As the rotary solenoid tried to retrieve the lines, one line was cut by one of the many sharp rocks on the ground; the other line pulled free of the projectile after considerable force was applied.

These difficulties with the projectiles were most disheartening, since essentially the same line-winding technique for the projectiles had shown such good success in the many tests performed in the Washington area. However, it should be noted that a very minor modification was incorporated into the winding of the projectiles used for these field tests. This modification involved a change in the tool which was used in hand winding the lines prior to their insertion into the projectiles. The minor change in this tool was expected to improve the uniformity of these hand-wound "bundles of string" and to eliminate a problem encountered a few times during the check-out preceeding previous tests. However, it might have introduced some insidious change which resulted in the poor performance of the projectiles.

The bi-metallic thermostat which controls the current to the heater for the uninoculated sterile control unit malfunctioned. It was the first time one of these termostats had been observed to stick in the closed position. This resulted in a constant heat input of fifteen watts into the incubation chamber. When it was discovered that the thermostat had stuck, the incubation chamber of the uninoculated sterile control unit was so hot it could not

be touched by hand. This apparently caused the nutrient broth to boil and the chamber to pressurize; because, when the unit was eventaully dis-assembled, the window of the geiger tube was broken and the window and gas collector were impaled on the anode inside the detector.

By 7:30 p.m., the snow storm was very severe and the equipment was loaded into the vehicles. Weather conditions did not permit returning to the quarters at the University of California station. Although the party was marooned on the mountain by the snow storm, it was anticipated that data could be continued to be taken since the system could be operated from the portable power supply. However, the battery for the power supply apparently was not completely charged and the output voltage of the supply dropped too low to operate the system properly.

After the party was rescued the following day, the equipment was again put into operation (approximately 26 hours after the test started). The results obtained after the first hour of operation were quite inconclusive since the instruments had been exposed to a wide range of temperatures in the back of the vehicle in which the eight men of the party spent the night, and the gas collectors may have been saturated with natural CO_2 since they were probably exposed to fairly high concertrations exhaled by these men. The gas collectors were changed the second morning following the initiation of the test. This second set of gas collectors was on the units for approximately another 24 hours, at which time the test unit showed about 18,000 cpm

and the inhibited 1800 cpm. The first set of gas collectors were saved and eventually checked for saturation. They were found to be saturated with untagged CO_2.

b. Sand dunes at the southeast edge of Death Valley. This test, conducted October 20, 1963, on the sand dunes about 35 miles north of Baker, California, was started shortly before 4:00 p.m. The air temperature was about 30°C and the temperature of the sand about 32°C. All mechanical operations were satisfactory, except for the projectiles. Only one of the four projectiles over-travelled the length of the line during its trajectory in air. The collection lines pulled out of all the projectiles satisfactorily as the lines were retrieved. The three projectiles were dragged varying distances in the loose sand, digging little furrows, before the lines pulled free. The amount of sand collected by the lines appeared to be fairly large.

An electronic problem appeared with the uninoculated sterile control unit. A 60-cycle signal was superimposed on the signals from the radiation detector. This was eliminated by cutting the leads to the heater of the unit. A new thermostat for controlling the heater current had been installed prior to this test since the previous thermostat had malfunctioned at White Mountain. Prior to leaving for the field test there had been no difficulty with the 60-cycle signal when the unit was checked out in the laboratory using 60-cycle line power, and this difficulty in the field was most unexpected. The power for the heater is 28 VAC, stepped down from the 110 VAC from the

portable power supply. This AC power is square wave rather than sine wave. After returning from California it was determined that the problem resulted from greater capacitive coupling of the square signal through the heater and thermostat to the body of the instrument which was tied to a virtual ground along with the cathode of the geiger tube. Since the geiger signal was taken off the cathode, this capacitively coupled 60-cycle signal fed through the amplifier to the counter.

The count rate for the uninoculated sterile control was considerably higher than previous tests. Speculations as to the cause(s) of this high level are: (1) the uninoculated sterile control was not flushed with air as were the test units; (2) the ampoules had been sealed for more than a week; (3) the broth ampoule for this unit was dipped in alcohol and flamed twice prior to assembly of the unit since it was feared that the surface of the ampoule touched a nonsterile surface; and (4) when this ampoule was put in the unit the body of the unit was still so hot after coming out of the autoclave that it could not be held by hand and this heat may have decomposed some of the broth. The exact cause of this higher sterile control level will be determined by a series of laboratory tests now being planned by RRI and techniques for reducing the level have been proposed.

Probably the most perplexing difficulty with this test was that the antimetabolite was accidentally injected into the test unit. In examining the circumstances and activities to determine when the switch could have been thrown which injects the antimetabolite, it was concluded that it must have

happened during the return from the sand dunes to the motel at Baker, Calif. The switch for injecting the antimetabolite is on the programmer and is protected by a guard which requires lifting the guard before the switch can be thrown. It is speculated that this guard was lifted and the switch thrown as the programmer brushed against the seat of the sedan during loading. To prevent such a mishap during future tests, it was decided that the leads to the squib for firing the antimetabolite for the growth or test unit would always be cut as soon as the antimetabolite was fired in the other test unit to effect the inhibited control.

When the gas collectors for this test were checked for saturation they were found still to have good affinity for CO_2.

c. Desert near Salton Sea, California. This test was started about 1:00 p.m. October 24, 1963, with the air temperature about 31°C and the soil about 43°C in the sun. Again, with the exception of the projectiles, all automated functions were performed satisfactorily. Two of the projectiles performed properly. Part of the line was pulled free of one of the projectiles as the lines were retrieved, and with the fourth projectile the line broke during the trajectory of the projectile in air. It was the first time in all the tests of this type of projectile that a line has broken during the trajectory in air.

The line retrieval system on one of the test units operated intermittently. The lines were retrieved satisfactorily, but the cause of the

intermittent operation has not been positively identified. The suspected causes are that there was a bad transistor in the pulsing circuit for driving the rotary solenoid or there was a binding of the bearings in the bearing race in the solenoid.

The 60-cycle signal again was superimposed on the signal from the geiger tube of the uninoculated sterile control. Again, by cutting the heater leads, the 60-cycle signal was eliminated and the count rate restored to a normal level.

The count rate of the uninoculated sterile control again rose to a fairly high level before it leveled off. Since the broth was not subjected to excessive heat, as was the situation with the previous test, it was speculated that this high sterile control resulted from the fact that there was no flushing of the broth prior to sealing the incubation chamber and that the ampoules had been sealed for nearly two weeks.

The gas collectors were found still to have good affinity for CO_2 when they were checked for saturation after being on the test units for about 26 hours.